show similar behavior. They promote the adsorption of vesicles to an air/water interface by a process analogous to fusion. They enhance the negative spontaneous curvature of H_{II} monolayers formed by dioleoyl phosphatidylethanolamine (DOPE):dioleoyl phosphatidylglycerol (9:1, mol:mol), but only by 8%. The studies here tested whether the limited response might reflect the size of the H_{II} cylinder and the restricted space available for incorporation of the proteins. Small angle x-ray scattering showed that different ratios of DOPE with dioleoyl phosphatidylcholine (DOPC) produced H_{II} cylinders with different radii. The effects of the proteins depended on the cylindrical size. Up to 3% (w:w) of the proteins produced no change in the smallest cylinders, formed by DOPE alone. With larger cylinders, the proteins reduced the radius. The magnitude of the change increased with cylindrical size. At 80% (mol:mol) DOPC, 5% (wt:wt) protein reduced the outer cylindrical radius by 78%. This change represented a 90% increase in the estimated magnitude of negative spontaneous curvature. These results show that demonstrating the ability of the hydrophobic surfactant proteins to promote negative curvature requires H_{II} cylinders above a minimum size. Our findings raise the possibility that the effects on H_{II}-curvature of other fusogenic peptides might also depend on the cylindrical radius. Acknowledgements: NIH; SSRL.

1509-Pos Board B239

Dengue Virus Capsid Protein Delivers Nucleic Acids Intracellularly

Miguel A. Castanho[1], João Freire[1], A. Salomé Veiga[1], Thaís Conceição[2], Wioleta Kowalczyk[3], Ronaldo Borges[4], David Andreu[5], Nuno Santos[1], Andrea Da Poian[6].
[1]Inst Molecular Medicine, School Medicine, University Lisbon, Lisbon, Portugal, [2]Inst Bioquimica Medica, UFRJ, Rio Janeiro, Brazil, [3]Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, Spain, [4]UFRJ, rio Janeiro, Brazil, [5]Department of Experimental and Health Sciences, Universidade Pompeu Fabra, Barcelona, Spain, [6]Instituto Bioquimica Médica, UFRJ, Rio Janeiro, Brazil.

Supercharged proteins are a recently identified class of proteins that have the ability to deliver functional macromolecules into mammalian cells very efficiently. They were first known as bioengineering products but were later found in the human proteome. In this work we show that this class of proteins with unusually high net positive charge is frequently found among viral structural proteins, more specifically among capsid proteins. In particular, the capsid proteins of viruses from of the *Flaviviridae* family have all a very high net charge/molecular weight ratio ($> +1.07$/kDa), thus qualifying as supercharged proteins. This ubiquity raises the hypothesis that supercharged viral capsid proteins may have biological roles that arise from an intrinsic ability to penetrate cells. Dengue virus capsid protein was selected for a detailed experimental analysis. We showed that this protein is able to deliver functional nucleic acids into mammalian cells. The same result was obtained with two isolated domains from this protein, one of them being able to translocate lipid bilayers independently of endocytic routes. Nucleic acids such as siRNA and plasmids were delivered fully functional into cells. It is possible that the ability to penetrate cells is part of the native biological functions of these proteins.

1510-Pos Board B240

Towards PHLIP Insertion in the Plasmamembrane of Cancer Cells at Physiological Tumor Acidity

Ming An[1], Joab Onyango[1], Michael S. Chung[1], Raemer J. Lapid[1], Emma A. Gordon[1], Rachel Langenbacher[1], Syris Winge-Barnes[1], Rebecca A. Chandler[1], Donald M. Engelman[2], Lan Yao[3].
[1]Chemistry, State University of New York, Binghamton University, Binghamton, NY, USA, [2]Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, [3]Physics, Applied Physics and Astronomy, State University of New York, Binghamton University, Binghamton, NY, USA.

The pH-(Low) Insertion Peptide (pHLIP) can target cancer cells based on their low extracellular pH (pHe). Under slightly acidic conditions, pHLIP inserts into membrane, forming a transmembrane (TM) helix. When cargo is attached to pHLIP's C-terminus, it is carried across the membrane during pHLIP insertion. Therefore, pHLIP is also a drug carrier that can deliver cargo directly into the cell cytoplasm. Imaging studies have shown that pHLIP can target acidic tumors in mice (and the signal stayed in the tumor for more than a week). We ask whether such targeting and long residence time arise from pHLIP insertion into the plasmamembrane of cancer cells or preferential binding to cell surface without TM insertion (followed by endocytosis and insertion in the endosomal membrane). To quantify pHLIP insertion in cells, we developed fluorescently self-quenched pHLIP probes that upon interactions with cells become more fluorescent. The turn-on fluorescence (i.e. dequenching) results from cleavage of a C-terminal disulfide bond that would only occur in cells. Our data suggest that even at pH 6.2 (which is more acidic than tumor pHe), pHLIP insertion is highly cell line dependent. When the experiment was carried out at 4°C instead of 37°C, about 50% of the gain in fluorescence signal is lost, suggesting at least half of pHLIP insertion occurred in endosomes. Further, compared to the 'WT' pHLIP, the D25E pHLIP variant inserts more readily at plasmamembrane. Using Structure-Activity-Relationship (SAR) studies (i.e. by changing pHLIP sequence and/or side-chain structures), we searched for new pHLIP variants that would (a) insert in plasmamembrane more readily at the tumor acidity of pH 6.5-7.0, and (b) with insertion occurring over a narrower pH range. The results of these SAR efforts will also be discussed.

1511-Pos Board B241

Structural Insights into Human Hemokinin1 -Nk1Receptor Interactions

Anjali M. Ganjiwale[1], Priyanka Mishra[2], Deepak Bhatnagar[3], **Sudha M. Cowsik**[4].
[1]Institute of Bioinformatics and Applied Biotechnology, Bangalore, India, [2]School of Biochemistry, Devi Ahilaya University, Indore, India, [3]School of Biochemistry, Devi Ahalaya University, Indore, India, [4]School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.

PPT-C encoded hemokinin-1 (TGKASQFFGLM) of Homo sapiens is a structurally distinct neuropeptide among the tachykinin family that participate in the NK-1 receptor downstream signaling processes. Subsequently, signal transduction leads to execution of various effecter functions which includes aging, immunological and central nervous system (CNS) regulatory actions. However the conformational pattern of ligand receptor binding is unclear. The three-dimensional structure of the hemokinin-1 in aqueous and micellar environment has been studied by CD Spectroscopy and two-dimensional proton nuclear magnetic resonance (2D 1H-NMR spectroscopy) and distance geometry calculations. CD data shows that hemokinin-1 was unstructured in aqueous environment; anionic detergent SDS induces α-helix formation. Proton NMR assignments have been carried out with the aid of correlation spectroscopy (gradient-COSY and TOCSY) and nuclear Overhauser effect spectroscopy (NOESY and ROESY) experiments. The inter proton distances and dihedral angle constraints obtained from the NMR data have been used in torsion angle dynamics algorithm for NMR applications (CYANA) to generate a family of structures, which have been refined using restrained energy minimization and dynamics. The results show that in aqueous environment hemokinin-1 lacks a definite secondary structure. The structure is well defined in presence of dodecyl phosphocholine micelles. The conformational range of the peptide revealed by NMR and CD studies has been analyzed in terms of characteristic secondary features. Observed conformational features have been compared to that of Substance P, Neurokinin A and Neurokinin B, potent NK1, NK2 and NK3 agonists respectively. Thus the report provides a structural insight to study hHK-1-NK1 interaction that is essential for hHK1 based signaling events.

1512-Pos Board B242

Peptide Lipidation by Acyl Transfer from Membrane Lipids and Lyso-Lipids

Vian Ismail[1], Burkhard Bechinger[2], Jackie A. Mosely[1], **John M. Sanderson**[1].
[1]Dept. of Chemistry, Durham University, Durham, United Kingdom, [2]Institut de Chimie (UMR-7177), University of Strasbourg, Strasbourg, France.

Acyl group transfer to membrane-active peptides (exemplified by melittin, magainin II, PGLa and penetratin) occurs following their addition to phospholipid membranes composed of phospholipids (doi: 10.1016/j.jmb.2013.07.013). This chemical reactivity, termed *intrinsic lipidation*, exhibits modest selectivity according to the lipid composition of the membrane. Transfer is favored in mixtures of PC with PS and PG. Mixtures of PC with PE exhibit more complex patterns of reactivity. *Lyso*-PCs are also able to act as acyl group donors. The intrinsic lipidation of melittin occurs below the threshold peptide to lipid ratio for toroidal pore formation and over physiologically significant ranges of temperature (20-37 °C) and ionic strength (0-150 mM NaCl). Intrinsic lipidation is able to generate significant chemical complexity from a small number of components. As lipidation induces significant changes to the structure and membrane affinity of peptides, this process has significant implications for any procedure that involves peptide addition to lipid membranes. In addition to accounting for behavior such as irreversible binding in peptide-lipid binding experiments, intrinsic lipidation provides an explanation for a number of unusual lipidation patterns found in membrane proteins.